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EFFECTS OF CHEMICAL VARIATIONS IN AQUATIC ENVIRONMENTS. VOLUME II. TOXIC EFFECTS OF AQUEOUS ALUMINUM TO RAINBOW TROUT

W. Harry Everhart, et al

Colorado State University

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Effects of Chemical Variations in Aquatic Environments Vol. II Toxic Effects of Aqueous Aluminum to Rainbow Trout

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EFFECTS OF CHEMICAL VARIATIONS IN AQUATIC ENVIRONMENTS:

Volume II

Toxic Effects of Aqueous Aluminum to Rainbow Trout

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ABSTRACT

Fertilized eggs, fry, and fingerlings were exposed to aqueous aluminum complexes in neutral and basic media under constantly flowing, controlled conditions of aluminum concentration, pH, and temperature. Toxicities of various concentrations were highly pH dependent. Dissolved concentrations over 1.5 ppm aluminum caused physiological and behavioral aberrations as well as acute mortality. Toxic effects of suspended aluminum, though greater at lower concentrations, do not increase as much as the effects of dissolved aluminum with higher concentrations. Growth of trout exposed to high dosages of aluminum was reduced only as long as or slightly longer than the exposure continued.

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SECTION I

CONCLUSIONS

1. Dissolved and suspended forms of aqueous aluminum are toxic to rainbow trout.

2. Debilitated condition of fish after only a week's exposure to 5.2 ppm aluminum at any pH tested would have made it difficult for them to withstand a flow rate characteristic of a trout stream or river during such activities as migration, feeding, or escaping from predators.

3. Extremely acute mortalities occurred at 5.2 ppm dissolved aluminum while mortality rates were more chronic with equivalent suspended amounts. Effects on activity and coloration were more pronounced in dissolved concentrations. Both forms advanced gill hyperplasia to the point of near total fusion of filaments.

4. At 0.52 ppm the occurrence of first symptoms of distress did not appear until after three to six weeks of exposure. Eventual mortalities were much higher in number at pH 7.0 (44%) than at 8.0 (8%) where all aluminum was soluble.

5. At 0.05 ppm of dissolved aluminum, growth was excellent and behavior normal. It is evident that this concentration can be tolerated without any obvious effects on growth or behavior. This amount represents saturation at pH 7.0 and is totally soluble at all higher pH's.

6. Mortality rates at 5.2 ppm at pH 7, 0.52 ppm at pH 7, and 5.2 ppm at pH 8 were all similar indicating toxic effects begin at lower concentrations of suspended aluminum than dissolved aluminum. The concentration dependency of suspended aluminum toxicity is of very low order while soluble toxicities are strongly concentration dependent.

7. Loss of appetite, greatly decreased activity and gill hyperplasia initiate sooner at 5.2 ppm than 0.52 ppm (pH 7 and 8), but after six weeks there was little difference between the fish of the two groups.

8. Neither brief nor extended contact with lethal amounts of both soluble and/or insoluble aluminum produces irreversible physiological damage as far as growth is concerned to young trout surviving the exposure.

Fertilization and Early Life History

9. Comparison of mortalities from egg lots fertilized in the aluminum concentrations and in the control water did not show any significant advantages or disadvantages to either.

10. At pH 9.0 only the 5.2 ppm aluminum concentration demonstrated a higher mortality than the control.

11. Aluminum concentration of 5.2 ppm at pH 8.0 demonstrated the highest overall mortality. Aluminum at 5.2 ppm and pH 7.0 was next toxic. Twenty-nine percent of the pH 8.0 mortality occurred at hatching when young rainbows died just as the embryo was breaking through the shell. At pH 7.0 eight percent of the mortality occurred just as the head of the embryo emerged from the egg shell.

SECTION II

RECOMMENDATIONS

General

Basic chemical research is needed to produce more accurate methods of chemical analysis in natural waters. Much is lost with instruments capable of precise determinations if we don't know the form of the chemical or if natural complexes interfere with analysis.

The problem of recommending safe concentrations for aluminum is much more difficult than for most other metals. Methods of determining aluminum in solution are commonly insensitive to colloidal precipitates. The aluminum methods proposed by Standard Methods are additionally somewhat insensitive to polymeric aluminum hydroxide ions. Atomic absorption methods are less sensitive to aluminum than other common metals.

In the practical sense, the enforcement of regulations regarding permissible aluminum concentrations will be very different since toxic effects occur at concentrations that will be difficult to analyze in unknown waters where interference also adds to detection problems. It is certain, however, that the old rule of permitting 1/10 of the 96 hour TL_{50} concentration will not hold. Under some conditions 1.5 ppm aluminum would be more toxic than 5 ppm under other conditions. The effect of complexation by ligands other than hydroxide is, as yet, another total unknown.

If hydroxide complexed aluminum is present only as anionic and neutral or near neutral precipitated forms, a condition that should hold for most natural waters with pH greater than 5.5, tolerable concentrations of either form probably should not exceed 0.10 ppm if rainbow trout are to survive and grow normally.

SECTION III

INTRODUCTION

A real need in the management of our water resources is basic information on which to base recommendations for establishing water standards or criteria for pollutants. The importance of our sport and commercial fisheries emphasizes the need for detailed studies of the effects of toxicants on the fish populations. Among the objectives of this grant were to investigate the toxicity to rainbow trout (Salmo gairdneri) of soluble anionic species and neutral precipitates of aluminum hydroxide and lead nitrate complexes under known conditions of pH and concentration. The aluminum studies were divided into effects of aluminum on fingerling rainbows and effects of aluminum on fertilization and early life history.

Aluminum is abundant in the earth's crust, but its natural occurrence is limited to highly insoluble complex minerals. As a result, the concentration of aluminum in natural waters is minute. The importance of aluminum as a pollutant has been reemphasized by the impending development of oil shale mining in several Rocky Mountain States. The liberation of aluminum from Dawsonite ($NaAl(OH)_2\ CO_3$, which is present in some oil shale ore, is a biproduct of some oil shale mining processes.

Behavior of aluminum in aqueous solution is extremely complex; it forms a variety of complexes with water, hydroxide, fluoride, silicate, and sulfate. The free aluminum ion is rare occurring only in very minute amounts. Possible forms exhibit vastly different structures and activities and will certainly be found to produce different effects on living organisms.

The chemical nature of aluminum in dilute solution is controversial. Slow reaction rates, the formation of metastable solids and gels, colloidal and subcolloidal suspensions, and marginal analytical techniques add to the problems of determining water associated structures. The reports of Hem and Roberson (1967), Hem (1968) and Roberson and Hem (1969) provide lucid explanations of the behavior of aluminum in natural waters.

Solubility of aluminum under conditions where hydroxide complexes dominate (the vast majority of natural waters) is, briefly, a direct function of pH, decreasing toward 5.5 and increasing toward both extremes of the pH scale (Figure 1). Soluble forms in acidic environments are polymeric and cationic while monomeric anions are present in basic media. The critical importance of pH is exemplified by its control of complexation, polymerization, hydrolysis, and solubility.

In acidic conditions, monomeric hydrolysis of the hexaquo complex is followed by the joining of two such monomers to form a dimer, sharing the two OH ions on a common edge and eliminating two water molecules (Hem, 1968).

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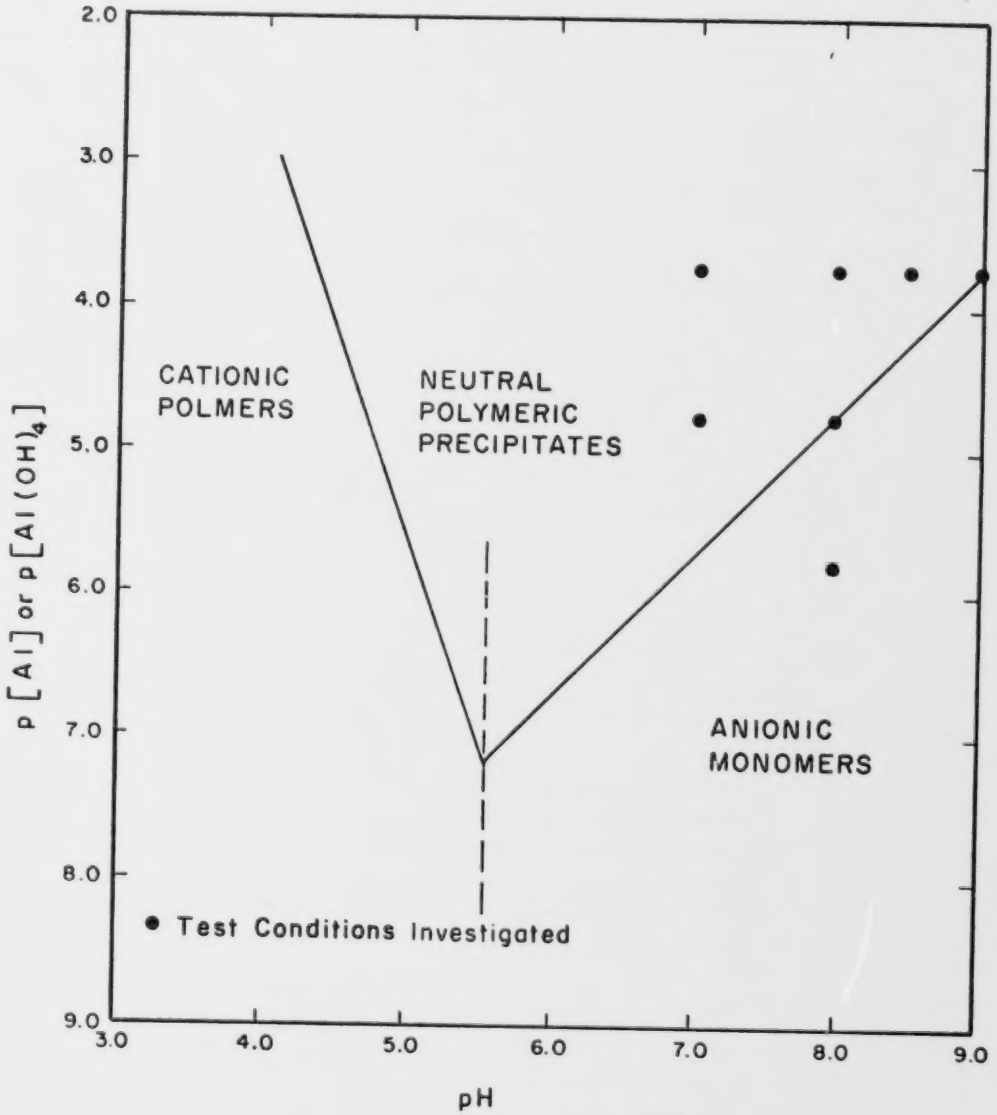

FIG. 1 SOLUBILITY OF ALUMINUM HYDROXIDE COMPLEXES (Modified from Hem, 1967)

This shared edge is the basic building linkage in the continuing process of polymerization with no apparent limit on polymer size. As the polymer enlarges, chains and then six membered planar rings of complexes are formed. The ring systems become less soluble as the polymers enlarge. The basic complexes retain a monomeric configuration but hydrolize at four sites to produce the anion, $(Al\ (OH)_4(H_2O)_2)^-$.

Previous investigations into aluminum toxicity have shown little regard for aluminum's complex chemistry, resulting in widely divergent results. For example, Jones (1964) reported that 0.07 ppm was the limiting safe concentration of aluminum to sticklebacks whereas Schaut (1939) stated that as long as aluminum did not result in critically low pH's (via hydrolysis) it should have no toxic effects on river fish. Many studies have utilized conditions which exceeded the solubility of aluminum with no account of the precipitates. Others have investigated conditions where pH could have been a lethal factor.

Previous studies with fish include Thomas (1915), Ebeling (1928), Oshima (1931), Ellis (1937), Sanborn (1945), Minkina (1946), Pulley (1950), Wallen et al (1957), and Jones (1964).

IV METHODS

The bioassay apparatus was of constant flowing design. Principal components were eight troughs, three aluminum administering devices, a hydroxide diluter, heating and cooling units, and a pressure regulating reservoir (Figure 2).

Water for the system was tapped directly from a 10" municipal main. All piping for the system was either rigid or flexible polyvinyl chloride except for one section of steel pipe carrying high temperature water from the heaters to a temperature control valve. It was also necessary to use brass gate valves in this section (See Table 1). All other valves and fittings were plastic or polyethylene.

Two heaters connected in series with 110,000 BTU/hr were used to heat a portion of the water to about 60°C for eventual mixing to attain a temperature of 13°C (55°F) for the experiments. Mixing was achieved with a Powers 440 photopanel temperature control valve. To prevent supersaturation of the heated water, the input to the pressure regulating reservoir was splashed vigorously and directly so as to keep the water swirling and liberate excess gases. When ambient water temperature exceeded 55°F, the entire flow was passed through two 1 H.P. Min-o-cool refrigeration units.

All water was passed through two activated carbon filters connected in series for dechlorination. The piping of the dechlorinators was arranged to permit the removal and refilling of one unit without interrupting flow to the system.

The pressure regulating reservoir was equipped with a 1 1/2" diameter standpipe which was constantly just overflowing. This assured a constant head to the system and constant flow through the hydroxide diluter and to the troughs.

At full scale operation, the system required about 22 liters per minute: Two liters per minute per trough, a small portion of which came from the hydroxide diluter which required 3.5 liters per minute, and one liter per minute diverted from between the dechlorinators to a 3-gallon tank containing several fingerlings. These fish were used to monitor the performance of the first dechlorinator. As long as there was no mortality in this tank, the first dechlorinator was effectively removing chlorine and the second dechlorinator was not being consumed. The remainder of the 22 liters overflowed the standpipe of the pressure regulating reservoir.

The aluminum stock solution was dosed to the system by three constant flow devices, one per set of duplicate conditions, described by Freeman (1971).

A constant flow was metered into the hydroxide diluter where it was mixed with filtered concentrated NaOH solution metered from a 2-liter Mariotte

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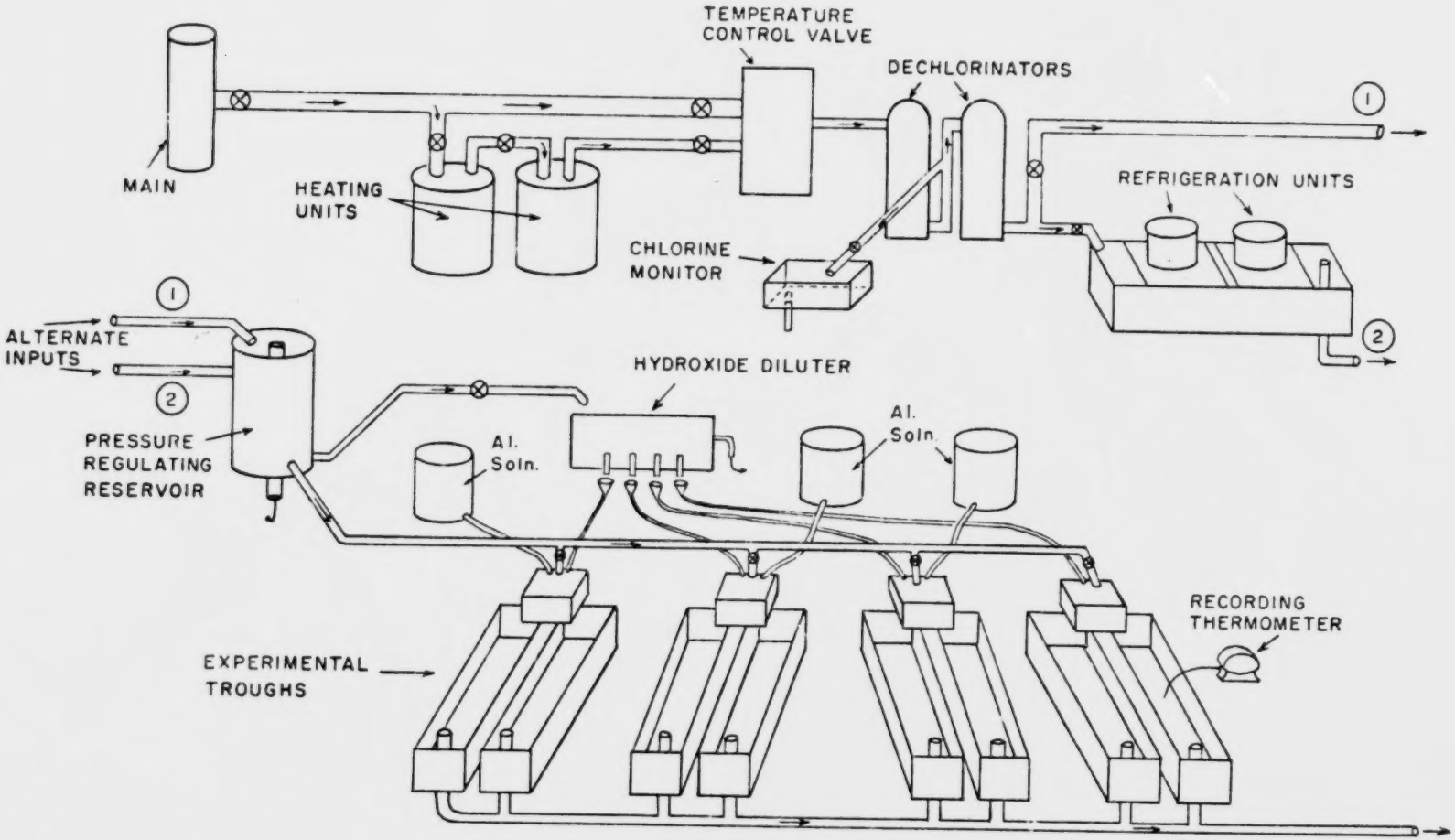

FIG. 2. SCHEMATIC DIAGRAM OF EXPERIMENTAL APPARATUS

bottle. The diluter was constructed of 6.35 mm (1/4") plexiglass, forming a rectangular trough about 35 cm x 6.2 cm x 7.5 cm. Standpipes of soft glass tubing inserted in rubber stoppers were placed in appropriate holes in the bottom of the trough of the diluter. The effluent from each standpipe was collected in a glass funnel suspended below the diluter and carried via rubber tubing to the appropriate trough. Two baffles at the head of the diluter induced turbulence to assure uniform mixing of the concentrated NaOH. The flow from the standpipes could be regulated by diameter of the tube and depth of placement. Enough water was passed through the diluter to maintain a constant level in its trough at all settings of the standpipes. At times, small plexiglass filter boxes packed with glass wool were placed over the smaller standpipes to filter particles of iron hydroxide precipitated from the water supply (Table 1) by the high pH of the diluter. This prevented adhesion of the iron hydroxide to the inside surfaces of the standpipes and the resulting disruption of desired constant flow rate.

The reagents were mixed and diluted in large orifice boxes at the head of each set of duplicate troughs. Each box was fitted with a perforated vertical and sloped horizontal baffle to induce turbulence and insure uniform dispersion of the reagents. Holes on opposite sides of the boxes delivered equal amounts of test solution to each of the duplicate troughs. Each box was calibrated to insure maintenance of correct dilution and flow rate. The remainder of the 2 liters per minute per trough was drawn directly from a manifold connected to the pressure regulating reservoir. Turnover time for the troughs was 69 minutes.

The fiberglass troughs measured 5' x 1' x 1' (152.4 cm x 30.5 cm x 30.5 cm). A mean depth of 29.7 cm was maintained by a standpipe at the end of each trough. A perforated weir blocked the standpipe from the rest of the trough, and 1/4" mesh screens were placed over the troughs to prevent fish from jumping out.

A sensor of a Foxboro recording thermometer was placed midway in one trough to monitor the temperature of the system.

A Corning Model 12 pH meter, graduated to 0.005 units, was used to monitor pH and a Gallet 7 jewel stopwatch graduated to 1/10 second was used to monitor the various flows of the system.

The NaOH was a purified flake from J. T. Baker and Co. The aluminum for the system was supplied by anhydrous aluminum chloride from Allied Chemical Co.

This study was designed to examine the toxic effects of aluminum at different pH's and concentrations. Concentrations chosen were significant in terms of possible solubility within the range of pH to be investigated. The range of conditions investigated and corresponding behavior of the aluminum were:

Total Aluminum Added	pH 7.0	pH 8.0	pH 8.5	pH 9.0
5.2 ppm	1% dis 99% sus	10% dis 90% sus	32.% dis 68% sus	100% dis
0.52 ppm	10% dis 90% sus	100% dis	100% dis	100% dis
.052 ppm	100% dis	100% dis	100% dis	100% dis

dis - dissolved sus - suspended

The mean solubility product prescribed by Hem (1967), $K_4 = 1.95 \pm 1 \times 10^{-13}$, was used to calculate the amounts of aluminum necessary to be added to achieve desired conditions, as well as those forms present under observed conditions. The rapid turnover time of the experimental tanks plus the movement of the fingerlings were sufficient to prevent significant settling of the precipitates at supersaturated conditions. When settling was observed it occurred behind the perforated weirs at the lower end of the troughs.

The procedure supplied by R. W. Smith and J. D. Hem (M.S.) involving the kinetic complexation of aluminum with ferron was employed to determine the various forms in which the aluminum species exists in aqueous media. The three forms described by Smith and Hem are the monomeric form, Al^a, described as Al^{3+}, $(Al(OH)_2)^+$ or $Al(OH)_4^-$, the polynuclear form, Al^b, described as a six-membered aluminum hydroxide ring, and the colloidal form Al^c, described as $Al(OH)_3$, aluminum hydroxide.

The only significant deviation from the procedure outlined by Smith and Hem was that anhydrous aluminum chloride was used to prepare the aluminum standard stock solution because of the difficulty encountered in preparing the solution from aluminum metal. This should not in any way affect the results and may in this case actually be desirable since the source of aluminum in the toxicity experiment was also anhydrous aluminum chloride.

Using this procedure on fresh solutions containing 5.2 ppm aluminum, the curves similar in shape to those described by Smith and Hem were obtained. The following data were then derived from the curves and the calibration curve using the methods described by Smith and Hem.

In general, this method entails extrapolation of the flat portion of the curve to zero time and estimating the amount of $Al^a + Al^b$ from the absorbance. The amount of Al^a is determined by the extrapolation of the curved portion of the curve to zero time. Al^c is determined by subtraction of $Al^a + Al^b$ from the total aluminum in solution, in this case 5.2 ppm.

Initial pH	Al^a	Al^b	Al^c	%Al diss*
6.0	1.1	2.2	1.9	21
7.0	1.3	2.4	1.5	25
8.0	1.8	2.4	1.0	35
9.0	4.0	0.8	0.4	77

*% Al dissolved - Al^a /Al total X 100%

Although they do not agree absolutely, the values for the percent dissolved aluminum as a function of pH follow the order of the calculated values.

Fluoride ion was periodically measured using a fluoride specific ion electrode and was consistently found to be very near 1.0 ppm, the value the City of Fort Collins claims to maintain. The method did not work at 0.52 ppm. Smith and Hem note that fluoride ion interferes with the analysis at low aluminum concentrations.

An interesting experimental result was observed by allowing the ferron to react completely with the aluminum species. In every case, regardless of pH, if the ferron-aluminum complex absorbance was measured 12-24 hours after the beginning of the experiment, all solutions were observed to have a final absorbance reading equivalent to a concentration of 5.1±.1 ppm. This measurement is representative of the total concentration of aluminum species in solution and agrees with the atomic absorption spectroscopy measurements in which the total aluminum concentration of solutions was found to be 5.0ppm±%.

Considering the difficulty in establishing steady state conditions in the troughs and the slow approach to equilibrium of the aluminum distribution, the agreement between calculated and experimentally determined aluminum distributions is better than might be expected. Either set of values illustrates that experimental conditions were chosen to span the range of distribution from dissolved to suspended aluminum.

Aluminum chloride was dissolved in tap water in the reservoirs of the constant flow devices. These devices were stopped for about 10 minutes for the refilling process. The hydrolysis of $AlCl_3$ results in highly acidic stock solutions. About 20 ml of concentrated hydrochloric acid was added per 200 liters of stock solution to insure rapid and total solution of the aluminum chloride when the reservoirs were refilled. Concentrations were adjusted so that either 18, 19, or 20 ml per minute, depending on which device was used, would supply the correct concentrations when diluted to two liters in the headboxes. Flows of these devices were monitored at three or four times daily during the experiment. The mean and variance of the concentrations of the experiments were calculated from these data. This was possible since all factors influencing test concentrations, namely concentrations of the stock solutions, delivery rates, and dilution rates were known.

Ambient pH of the input water plus the addition of various concentrations of the acidic aluminum solutions required that a basic additive be introduced to bring the test pH to the range desired and convert the cationic aluminum to its anionic form.

The pH of the system was controlled by dilute NaOH solution from the hydroxide diluter. A concentrated solution of 9.8 N NaOH was delivered at 1.4 ml per minute into 3.5 liters of water at the head of the diluter. Various amounts of sodium hydroxide were diluted via the diluter standpipes to bring pH's to test levels. The pH of the system was recorded three or four times daily and used to calculate the pH mean and variance of the system.

Data were taken from the continuous trace of the recording thermometer at three-four intervals to calculate mean and variance of temperature.

During the course of the tests, water samples were collected at about two week intervals for analysis of dissolved solids, dissolved oxygen, alkalinity, chloride, nitrate, silicate and sulfate (Table 1). Determinations were made by the procedures described in Standard Methods for the Examination of Water and Wastewater (1965). Metallic cations were determined by atomic absorption and emission spectroscopy.

Fingerlings

Limitations in laboratory size and available water made it necessary to raise the fingerlings in a water supply other than that used for the toxicity tests. Trout were hatched and reared to test size in well water and exposed to aluminum, after acclimation, in tap water (Table 1). All trout used in these experiments were hatched in the laboratory. Eyed eggs, spawned from infectious-pancreatic-necrosis-free brood stock, were superficially disinfected with Merthiolate and hatched in tray incubators. The eggs were hatched and fry raised to test size in well water. All fish used in the experiments had a recorded disease free history. Minor conditions of gill hyperplasia were prevalent when fish were held in well water. This condition rapidly disappeared when the trout were transferred to tap water for acclimation. Troughs holding the experimental subjects were cleaned daily. The well water supplied 10' x 1' x 1' (305 cm x 30.4cm) troughs at mean depth of 27.2 cm at a rate of 8 liters per minute. Fry and fingerlings were fed at all times according to a feeding chart modified from Deuel et al (1942). Clark's pelletized commercial trout chow was the food source.

Fry up to about a month old were fed six times per day. Older fry and fingerlings both before, during, and after testing were fed four times per day. Growth of all test animals was recorded at approximately two-week intervals and feeding rates adjusted according to growth.

Pretest weights were determined by weighing a sample of the pretest fingerlings. Weights of test animals during the test and during recovery were determined by weighing the entire group.

Table 1. Summary of water supply analyses

This water supply was used for the aluminum toxicity determinations. Concentrations are in mg/l.

Date	Dissolved Oxygen	Total Alkalinity[a]	pH[a]	Temp. (°C)[a]	Cl	NO_3	SO_4	$S:O_2$	
1/21/70	10.6	40.3	7.65	5.0	2.65	0.25	2.88	12.75	Tests at pH 8.0.
2/2/70	9.6	40.9	7.50	4.4	7.75	0.30	***	11.40	
2/21/70	10.0	40.9	7.60	3.3	3.50	0.13	***	10.80	
3/25/70	10.0	46.2	7.72	3.4	0.75	0.16	***	10.53	Tests at pH 8.5 & 9.0
4/13/70	9.0	50.6	7.75	7.2	2.50	0.20	***	12.30	
4/27/70	8.6	51.0	7.62	8.3	--	--	--	--	
5/7/70	8.8	39.3	7.44	11.7	1.50	0.22	***	12.20	Tests at pH 7.0
5/23/70	8.4	17.1	6.85	12.5	3.00	0.23	***	10.20	
6/16/70	9.2	13.6	6.90	12.9	--	--	--	--	

-- Not determined.
a Before adjustment.
* Less than 0.05 ppm.
** Less than 0.01 ppm.
*** Not detectable.

Continuation of Table 1

Date	Ca (2)	Mg (10)	Na (10)[a]	K (1)	Al (1)[a]	Fe (10)	Zn (1)	Cu (1)
1/21/70	16	5.0	2.9	4.4	*	--	--	--
2/2/70	20	1.0	1.0	*	*	0.20	**	*
2/21/70	10	0.2	2.0	*	*	0.20	**	*
3/25/70	11	0.2	1.1	*	*	0.15	**	*
5/7/70	22	0.4	4.5	*	*	0.45	**	*
5/25/70	22	0.4	4.5	*	*	0.45	**	*

Parentheses indicate confidence factorsfor data, i.e. the true value lies between that reported multiplied by or divided by the factor in parenthesis.

Test animals were transferred without tempering from the well water to the tap water for pretest acclimation. Fish for all tests, except at pH 7.0, were acclimated to tap water and pH for 8 days prior to introduction to test conditions. At pH 7.0 acclimation was limited to 3 days. In all cases, fish adapted to the change in water without any noticeable behavioral or physiological problems. Mortalities during the acclimation never exceeded 1%. During this time the pH was raised at the rate of 0.2 units per day to the test pH. At the end of the acclimation, healthy trout were selected from this group and placed directly into the various test conditions.

Behavior, growth, and mortality were recorded. Controls were weighed once or twice during the test to adjust feeding rates. The rates for all trout were then adjusted according to the growth of the controls rather than weighing the test fish and possibly altering mortality rates. Test fish were fed the prescribed food amount or as much as they would consume daily, whichever amount was smaller.

At the end of the exposures all groups were weighed. Some recovery subjects were transferred directly back to well water while others remained for a time in dechlorinated tap water. Growth of these fish was determined at about 10-day intervals by weighing the entire group. From these data the long term effects of aluminum on growth could be determined.

Time of death, weight of fish at death, and trough number for each mortality were recorded. From these data it was possible to determine the time of mortality of 50% of the group. Averages from the duplicates provided the reported TL_{50} time. Smirnov's statistic (Birnbaum, 1962), a non-parametric statistic used to compare two unknown distribution functions, was used to compare the empirical distribution functions of mortality rates between sets of duplicate conditions. The suspected dependence of survival time on size was also investigated by a simple regression; however, no significant relationship was discovered.

Fish were held prior to testing and for recovery in well water of the following analysis: D. O., 5.2-5.8 ppm; total alkalinity, 301-335 ppm; pH, 7.2-7.5; temperature, 16.5-16.7 C; chloride, 11-20 ppm; nitrate 2.9-3.2 ppm; sulfate, 62-86 ppm; silicate 14-17 ppm; calcium, 200-220 ppm; sodium, 125 ppm; potassium, 0.4-1.0 ppm; aluminum, iron, zinc and copper all less than 0.05 ppm.

Fertilization and very early life history

Eggs and sperm were collected from IPN certified free brood stock and fertilized in control water and in the aluminum concentrations. Fertilizing in control and treatment waters gave a chance to observe the effects of the aluminum on eggs, sperm, and the fertilized egg. In a further effort to approach natural conditions half of the eggs were reared in artificial redds of gravel and the other half in trays (Figure 3). An effort was

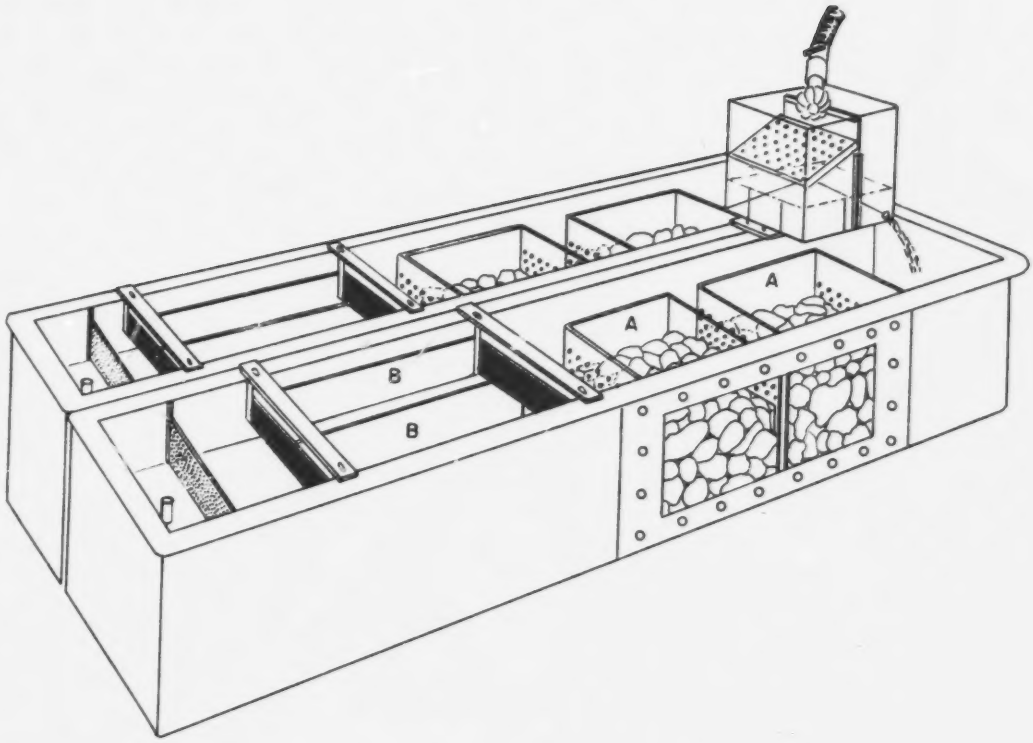

FIG. 3. ARRANGEMENT OF ARTIFICIAL REDDS (A, A) AND TRAY DIVIDED INTO TWO SECTIONS (B, B) IN TROUGHS.

made to deposit 200 eggs in each redd and in each tray. However, there was some variation in the actual number which was determined volumetrically to reduce any handling stress. Concern over the hatching success in the artificial redds dictated the use of trays. Dead eggs could not be removed from the eggs in the gravel and fungus could well have been the cause of loss rather than the aluminum. Eggs in the trays were examined daily and dead eggs removed.

In the first run, controls and experimentals were all at pH 9.0 with treatments of 0.052, 0.52, and 5.2 ppm aluminum. Replicates of each were provided. Half of the eggs were fertilized directly in the treatment water and the other half of the eggs were fertilized in the control water and then transferred to the treatment tanks, but separated in redds and trays.

In the second run controls were carried at pH 8.0, and the treatment tanks at pH 7.0 with aluminum concentration of 0.52 ppm, pH 7.0 and aluminum concentration of 5.2 ppm, and pH 8.0 and aluminum concentration of 5.2 ppm.

V RESULTS

Fingerlings

Aluminum Toxicity at pH 8.0

Duplicate concentrations of 0.05 ppm, 0.52 ppm, and 5.2 ppm aluminum were tested. Age of test fish was approximately 6 months at the start of the test. All exposures in this experiment were conducted for 45 days (1080 hours).

The control troughs received approximately 2.39 mg NaOH per liter to maintain a mean pH of 8.02 ($S_2 = 8.4 \times 10^{-3}$). No mortality occurred among the control fish for the duration of the test. These fish appeared healthy and active throughout the test.

At pH 8.0, 90% of the 5.2 ppm aluminum was insoluble. The insoluble aluminum hydroxide remained suspended in the troughs giving a slight whitish irridescent cast to the water. The coloration of slight accumulations of settled precipitate behind the weir at the downstream end of the troughs indicated that some iron was being coprecipitated by the aluminum hydroxide. The mean concentration of total aluminum added was 5.23 ppm ($s^2 = 0.051$). The mean pH for the test was 8.02 ($s^2 = 8.9 \times 10^{-3}$), thus a mean concentration of 0.55 ppm dissolved aluminum was maintained.

Within 24 hours an overall slowdown in activity was observed in the 5.2 ppm troughs, and almost all interest in feeding had disappeared. On no day of the test was more than half of the prescribed amount of food consumed. After five days gill hyperplasia was evident in many of the trout in both troughs. By the sixth day, many fish were experiencing momentary equilibrium problems or swimming on their backs breaking the surface, increasing in severity until the fish completely lost equilibrium and remained upside down for a period of from 3 to 24 hours preceding death. The coloration of these fish was very dark, many nearly black. During the second week of exposure, periods of increased activity and feeding occurred for durations of up to one day. After the fourteenth day, however, no increases in activity were ever observed. Overall physical condition steadily deteriorated with deaths occurring regularly. Within 30 days these fish lost all negative photoaxis to sunlight and all fright reaction to approach to the troughs by humans. Late in the test, trout could be prodded without attempting to flee. Respiratory rates were considerably higher than any other of the tests at pH 8.0.

After 45 days, weights of the 12 survivors averaged 30.2% of the control weight as opposed to averaging 106.5% of the control weight at the start of the test (Figure 4). Prior to release in well water, for recovery, experimental fish were tempered to it for a half hour. From this

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additional stress, however, 11 of the 12 died within 24 hours. The one survivor had returned to normal activity and feeding after six days.

A mean concentration of 0.514 ppm aluminum ($s^2 = 5.8 \times 10^{-3}$) was maintained at a pH of 8.03 ($s^2 = 7.2 \times 10^{-3}$)representing total saturation with soluble aluminum. Under these conditions, fish were slightly less active than the controls after a single day. After three days, a definite intermediate condition between the controls and the 0.52 ppm test existed. By the sixth day, consumption of food was considerably reduced. These fish consumed their total prescribed diet on about one half of the test days, feeding little if at all after 30 days. Darker coloration was evident in this test also, although not to the degree of the 5.2 ppm test. After 10 days exposure, there was a noticeable decrease in the fright reaction of the fish in these troughs. Periodic fluctuations in overall activity were observed throughout the test. Gill hyperplasia was evident in about half of the fish at 21 days. At the conclusion of the test, low power microscopic examination revealed that nearly all fish were showing hyperplasia to some degree.

Although only two deaths occurred very late in these tests, symptoms preceding mortality were identical to those exhibited by fish in the higher concentrations. Although these fish showed a net weight increase the average increase was 38% of the control increase. Their weights averaged 58.1% of the control weight following the exposures as opposed to 95.9% of the control weight at the start of the test (Figure 4). Continued exposure under these conditions would undoubtedly produce high mortalities.

At the conclusion of the test, five fish from each trough were placed without tempering into well water for recovery. Within 48 hours all symptoms of the exposure except the gill hyperplasia had disappeared. In the following 16 days, these fish showed extremely rapid weight increase comparable to the controls. No additional mortalities occurred and at the conclusion of the recovery period the fish were normal in all appearances.

A set of duplicate concentrations were also run at a mean concentration of 0.0516 ppm aluminum ($s^2 = 6.0 \times 10^{-4}$) at pH 8.04 ($s^2 = 3.3 \times 10^{-2}$). These conditions required the addition of 2.55 mg NaOH per liter to maintain pH. This amount of aluminum is the saturation point at pH 7.0 and is 100% soluble at all higher pH's.

These fish were healthy and active throughout the test, showing fast growth and normal behavior at all times. They averaged 101% of the control weight after the test as opposed to 97.2% of the control weight at the start of the test. Further testing of this concentration was not conducted since in the range of our investigations the form of this amount of aluminum would be unchanged.

Aluminum Toxicity at pH 8.5

At pH 8.5, about 3.19 mg NaOH per liter was necessary to maintain the control pH, while the test troughs required about 27.0 mg per liter. Fifty fingerlings were placed in each trough. Of the mean 5.14 ppm aluminum ($s^2 = 8.8 \times 10^{-2}$) added at mean pH 8.48 ($s^2 = 8.0 \times 10^{-3}$) a mean of 1.57 ppm aluminum

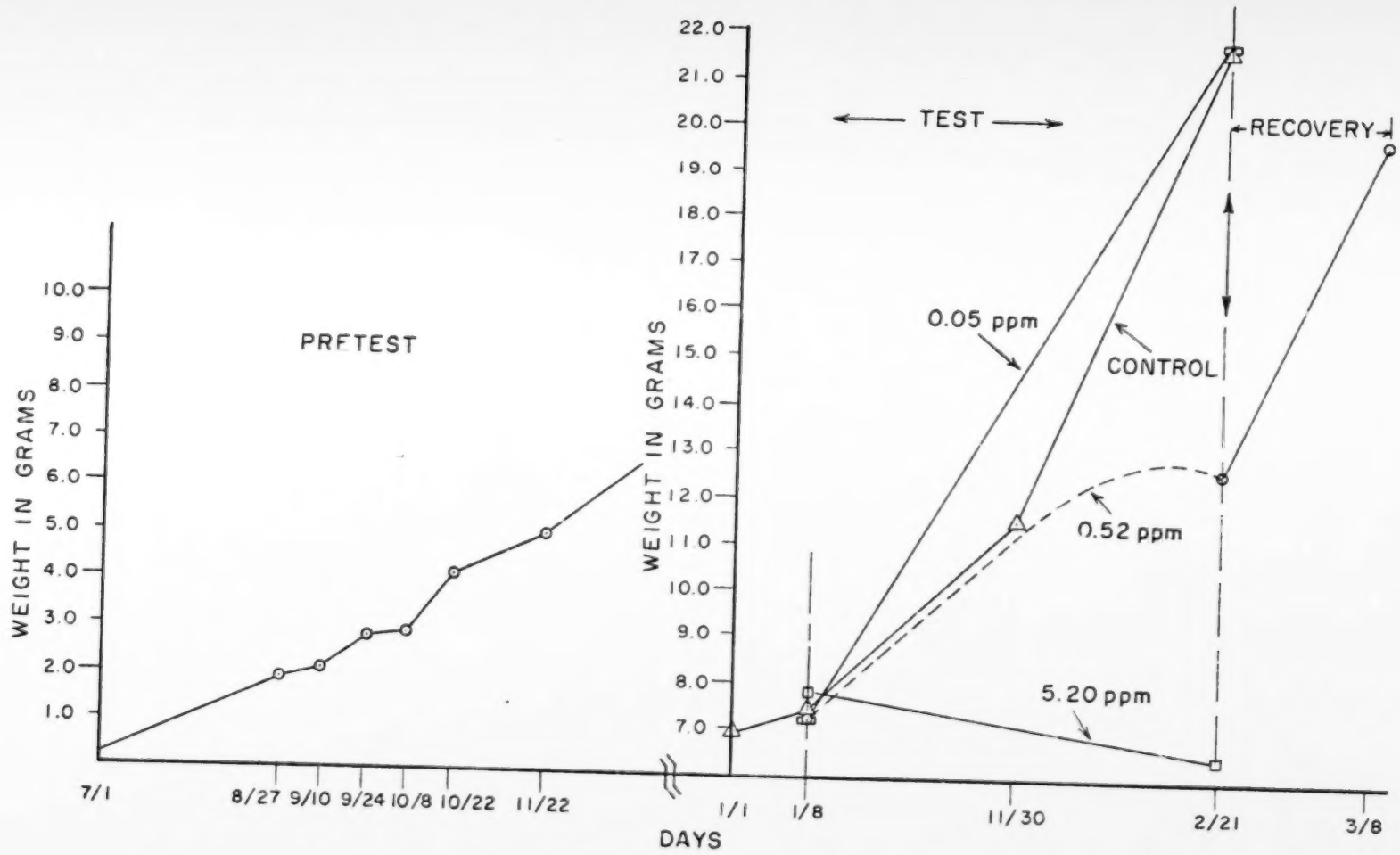

FIG. 4. GROWTH OF FINGERLINGS ASSOCIATED WITH ALUMINUM EXPOSURES AT pH 8.0.

was in solution. Trout used in these tests were about 6 weeks old at the beginning. Mean temperature for these tests was 12.6°C (s^2 = 0.14)

On introduction to the test conditions, fish showed immediate stress, darkening in coloration, and a total loss of interest in food. Mortalities began to occur almost immediately with all the symptoms previously described. In addition, fecal casts were obvious from all fish. This test was terminated after 222 hours (9 days 6 hours). Aluminum additions were stopped and pH was allowed to return to ambient, 7.7. Within 12 hours, about half of the fish had returned to normal coloration and began taking small amounts of food. After 72 hours, however, there was still no fright reaction in these fish. Overall normality seemed to have returned after five days, except for persisting gill hyperplasia. On the 8th day these fish were transferred without tempering into well water. For the next 10 days they showed no weight increase. After 18 days recovery, weighings indicated a near normal growth rate had resumed. At this time the weights of these fish averaged 55.2% of the control weight as opposed to averaging 106.9% of the control weight at the start of the test (Figure 5). After 49 days recovery they averaged 63.9% of the control weight. Mortalities during the first 60 days of recovery were 3% as opposed to 5% among controls. In the next 80 days there were no additional mortalities. After 140 days recovery, test fish averaged 82.3% of the control weight.

Aluminum Toxicity at pH 9.0

At pH= 9.0 approximately 5.07 mg NaOH per liter was added to maintain control pH while 35 mg per liter was required to maintain the test pH. The mean control pH was 8.99 ($s^2 = 5.5 \times 10^{-3}$). The mean test pH was 8.99 ($s^2 = 8.1 \times 10^{-3}$) with a mean aluminum concentration of 5.2 mg per liter ($s^2 = 8.0 \times 10^{-2}$). Under these conditions the mean concentration of soluble aluminum was 5.5 ppm (97.15%) and 0.15 ppm suspended (2.85%). Six week old fingerlings were used for this test. Mean temperature for the test was 12.6°C (s^2 = 0.18).

These fish showed immediate shock and heavy mortality within 48 hours. Fecal casts were wide spread and gill hyperplasia was universal within three days. No fish ever accepted food. Coloration was very dark and activity was minimal. Test conditions were terminated after 113 hours (4 days, 17 hours) by stopping the aluminum addition and allowing pH to return to ambient. Within 36 hours some fish began feeding and about one-half showed lightening of coloration. No fright reaction to approach appeared until after 5 days. By the sixth day, however, activity and feeding were near normal. On the eighth day some fish showed an apparent relapse as some test symptoms reappeared. On the tenth day the entire group was transferred to well water.

These fish also showed a loss in weight for the first two weeks of recovery resuming normal growth after 30 days recovery. Before testing, their weights averaged 116.75% that of the controls, but this dropped to 50.0%

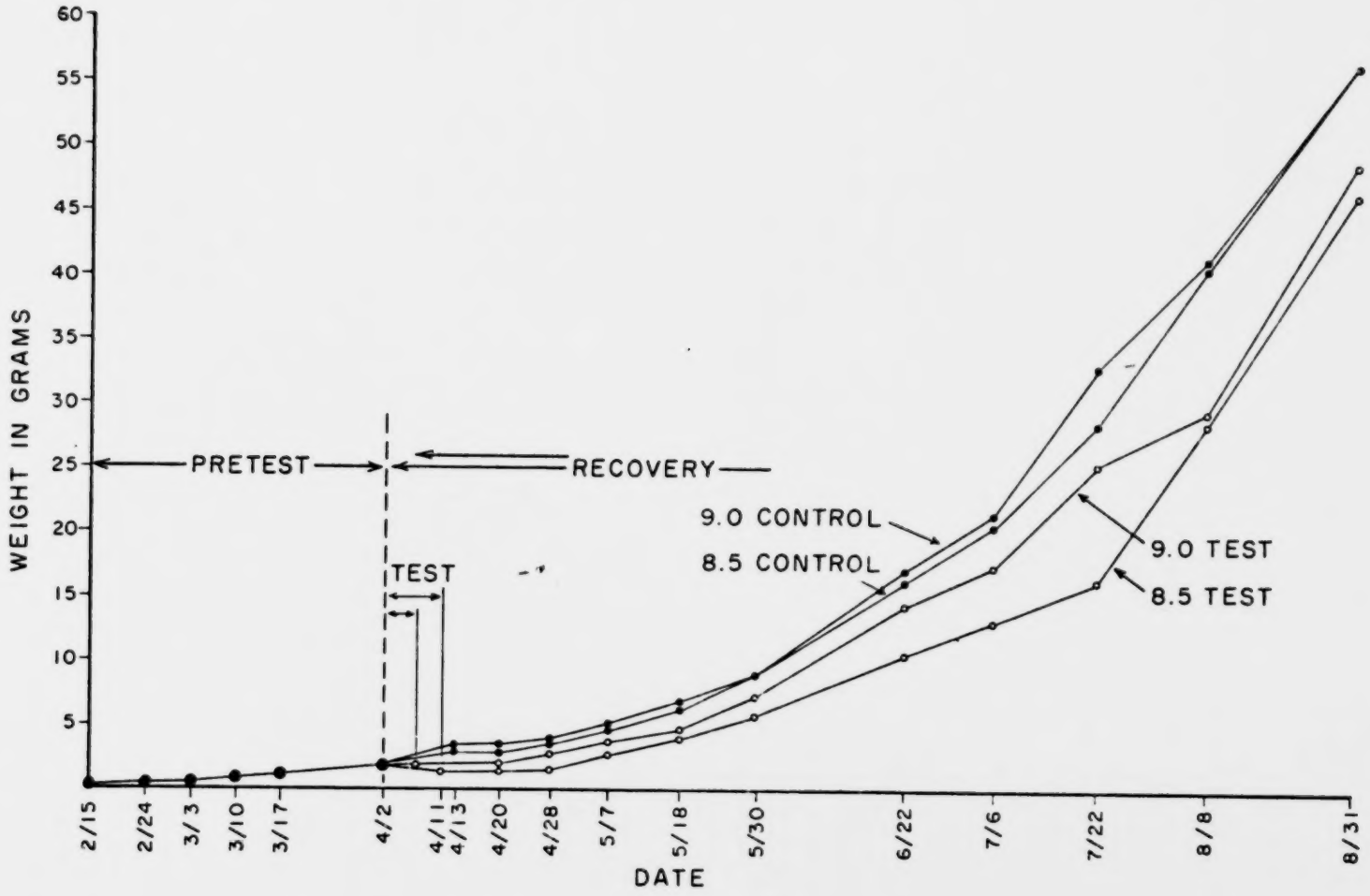

FIG. 5. GROWTH OF FINGERLINGS ASSOCIATED WITH ALUMINUM EXPOSURES AT pH 8.5 AND 9.0.

before climbing to 76.8% after 60 days recovery (Figure 5). During the first 140 days of recovery there were no mortalities among the recovering fish. After 140 days recovery, test fish averaged 85% of the control weight.

Aluminum Toxicity at pH 7.0

A final series of tests was carried out at a nominal pH of 7.0 At the beginning of these tests, the ambient input pH to the system was about 7.4. Since the pH controlling mechanism is suitable only for controlling pH's by either raising or lowering at a given time, not both simultaneously, it was decided to run the control and 0.52 ppm test without hydroxide addition and control only the 5.2 ppm test. The addition of 0.52 ppm aluminum did not reduce the pH of the system enough to permit control by hydroxide addition. At 5.2 ppm approximately 13.4 mg NaOH was added per liter for pH control. Solubility of aluminum in the pH range from 6.5 - 7.0 is practically constant so the absolute control of pH in this range is not nearly as critical as at a higher pH.

Complications were encountered when the water flow to the entire system became reduced for some unknown reason. As a result, the flow to the control troughs was reduced to 1.4 liters per minute for the test. All flow rates were in excess of that needed to support the quantities of fish used. No stress reactions were observed among the controls as a result of this adjustment.

On the seventh day of these tests, hydrated aluminum sulfate was introduced to the municipal water supply to precipitate the increased turbidity due to heavy snow melt run off in the Cache la Poudre River. These additions were continued for the duration of the test. According to treatment plant personnel, the alum was added empirically, but averaged 250 lbs. per million gallons of water. Such additions resulted in a total concentration of about 2.4 ppm aluminum with about 0.04 ppm soluble. Since the plant utilized large settling basins and is situated about 25 miles from the University, it is assumed that only the soluble portion was present in the input to the system. Such an amount is negligible in comparison to the additions of the tests themselves. Some curious behavior on the part of the control trout, namely refusal to feed, was noted on about 5 days of the test. The alum additions may be accountable for this. As a result of these additions, the input pH to the system fluctuated far more than normally. The pH of the 5.2 ppm test was difficult to regulate on this account.

The pH of the input water eventually became reduced to levels where the hydroxide diluter could have been used to raise the pH of the controls and 0.52 ppm tests. The solubility of aluminum is nearly constant in the region of the test pH. Sudden increases in the pH of the tests would expose the fish to conditions unlike those being tested if the input pH should arise sharply and hydroxide additions were continued, so no hydroxide was added. For the same reason, the pH of the 5.2 ppm test was held below

7.0. This precaution proved to be well founded as on several occasions the input pH increased to well above 7.0, and fish would have been exposed to acutely toxic conditions invalidating the test, had hydroxide additions been in progress.

The trout were eleven weeks old at the start of the test. All exposures were conducted for 45 days (1080 hours). The mean temperature for the test was 12.8°C ($s^2 = 0.28$). Twenty-five fish were used in each trough. The mean pH for the controls was 7.44 ($s^2 = 2.9 \times 10^{-2}$) for the first seven days and 6.85 ($s^2 = 4.4 \times 10^{-2}$) thereafter.

At a mean concentration of 0.513 ppm aluminum added ($s^2 = 6.3 \times 10^{-3}$), the pH was 7.27 ($s^2 = 2.8 \times 10^{-2}$) for the first seven days of exposure and 6.52 ($s^2 = 4.0 \times 10^{-2}$) thereafter.

Under these conditions, fish showed a darkening of coloration and reduced feeding activity beginning on the seventh day. Gill hyperplasia was observed on some individuals. These symptoms gradually increased in incidence and severity for the first five weeks of exposure. Mortalities began to occur on the twenty-third day and occurred regularly thereafter. At the conclusion of the exposure (45 days) these tests had not achieved 50% mortality. The average TL_{50} value reported for these conditions is the average of times projected by linear regression of the mortality rates. At the conclusion of the test these trout were feeding very erratically. Their average weight was 32.5% of the control average as opposed to 94.1% of the control average at the start of the tests (Figure 4). After 74 days recovery the average weight of test fish remains only 43.1% of the control average.

Another pair of duplicate exposures received a mean 5.14 ppm aluminum ($s^2 = 8.1 \times 10^{-2}$) at a mean pH of 6.80 ($s^2 = 6.8 \times 10^{-2}$). Under these conditions feeding stopped after four hours exposure. After four days, activity was drastically reduced and coloration was very dark. Gill hyperplasia was observed on all individuals within seven days. These trout exhibited a small degree of fright reaction throughout the test and began feeding very lightly for the last three weeks of exposure. Mortalities occurred at a regular rate throughout the test again preceded by a total loss of equilibrium. No fecal casts were observed.

Survivors of the test were extremely emaciated, averaging only 24.1% of the control weight as opposed to 91.8% of the control average at the beginning of the test (Figure 6). A single mortality occurred among the controls during this test. Resumption of growth among these fish proceeded very slowly as they averaged 29.5% of the control weight after 74 days recovery.

Smirnov's statistic was used to verify the agreement of mortality rates in sets of duplicate conditions. These tests indicated no significant difference among mortality rates of any set of duplicates ($a = .10$) except for the test at pH 9.0. Although by the conclusion of that study mortality

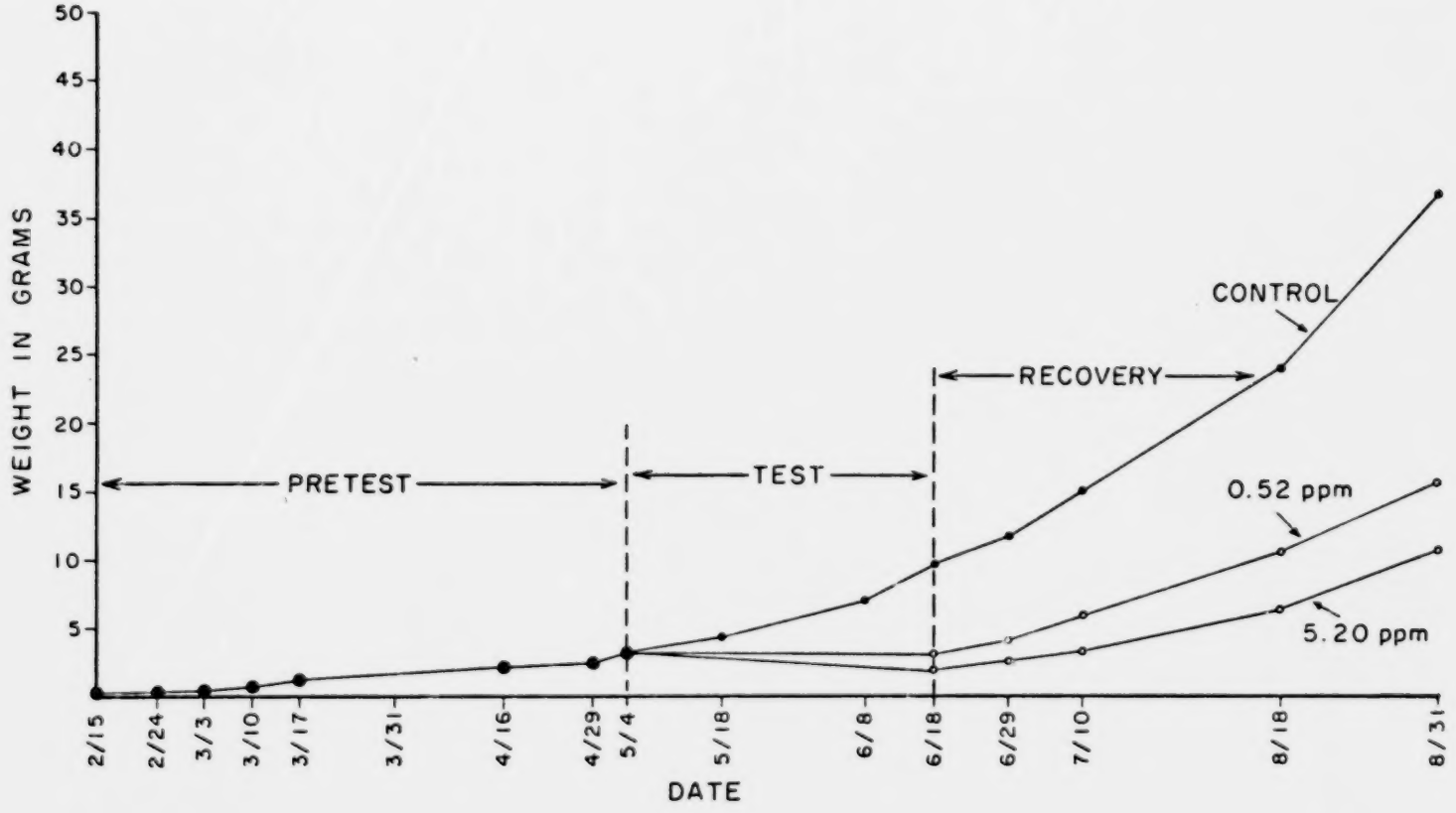

FIG. 6. GROWTH OF FINGERLINGS ASSOCIATED WITH ALUMINUM EXPOSURES AT PH 7.0.

rates were identical, early differences caused the tests to be significantly different (a =.01). The severity and short duration of this exposure are undoubtedly causing these variations.

As a result of noting the significant reduction in growth rates of the fingerling rainbows during the actual bioassay determinations experimental fish were maintained for several months following exposures of aluminum (Table 2).

Statistical analysis comparing the slopes of the recovery growth plots (Figures 7 and 8), as suggested by Dixon and Massey (1969) reveal no significant differences (α = .10) between growth rates of any group of test fish when compared to controls at the same weight (Table 3). The immediate implications of these findings suggest that neither brief or extended contact with lethal amounts of both soluble and/or insoluble aluminum will produce irreversible physiological damage as far as growth is concerned, to young trout surviving the exposure. Mortality rates returned to the control rate in all cases except for the test at pH 8, 5.2 ppm aluminum. In these conditions, 11 of 12 survivors succumbed during the first 24 hours of recovery, presumably due to their extremely debilitated condition, stress of handling, weighing and changing the water supply. Effects of maturation and fertility were not studied and may be significant.

Thus, the growth of trout exposed to high dosages of aluminum under these conditions will be reduced only as long as or slightly longer than the exposure is continued. In the case of a 45 day exposure, this means that the survivors are essentially 45-60 days behind in their development. In young fish this may easily account for a 100% weight difference even after an extensive recovery period. Applying this to natural situations, the size differential and subsequent delay in development could be important factors in susceptibility to predation, procuring available food, negotiating currents and spawning.

Fertilization and early life history

The research to study the effects of aluminum on the fertilization and early life history was designed to determine the toxicity by exposing eggs in simulated natural redds and in submerged screen trays (Figure 3).

Culturing the eggs in the simulated natural redds was unsuccessful as very heavy mortalities (80% to complete) indicated eggs and sac fry were additionally stressed by conditions (likely poor water flow through the gravel) in the redd along with the aluminum exposure. The few eggs that did develop to hatching lagged behind the eggs in the trays. Simulated natural conditions precluded disturbing the gravel for accurate counts at the eyed and hatching stages. Dead eggs could not be removed for the same reason, and fungus developed in all redds to provide an additional source of mortality. Likely a more sophisticated system of forcing water through the gravel would have improved the conditions.

Table 2. Summary of Recovery Growth Rates

Tests

pH Concentration	8.0 control	8.0 .52	8.0 5.2 ppm	8.5 control	8.5 5.2 ppm	9.0 control	9.0 5.2 ppm	7.0 control	7.0 0.52 ppm	7.2 5.2 ppm
Exposure time (days)	45.0	45	45.0	9.25	9.25	4.7	4.7	45.0	45.0	45.0
*Average weight start bioassay (gms)	7.4	7.1	7.9	2.0	2.2	1.9	2.2	3.4	3.2	3.1
*Average weight end bioassay (gms)	21.6	12.5	6.5	3.0	1.5	3.5	2.2	9.6	3.1	2.3
*Mortality during bioassay (%)	.0	8.0	77.0	0	51.0	.0	67.0	2.0	44.0	58.0
Average weight end recovery (gms)	30.0	17.5	-	73.5	58.3	73.5	65.3	205.9	109.0	96.4
Recovery time (days)	16.0	16.0	1.0	161.0	161.0	165.0	165.0	290.0	290.0	290.0
*+Mortality during recovery (%)	-	0	83	5	3	2	2	3	5	16

* Average of 2 replicates

+ Adjusted to allow for periodic random thinning of groups to prevent overcrowding

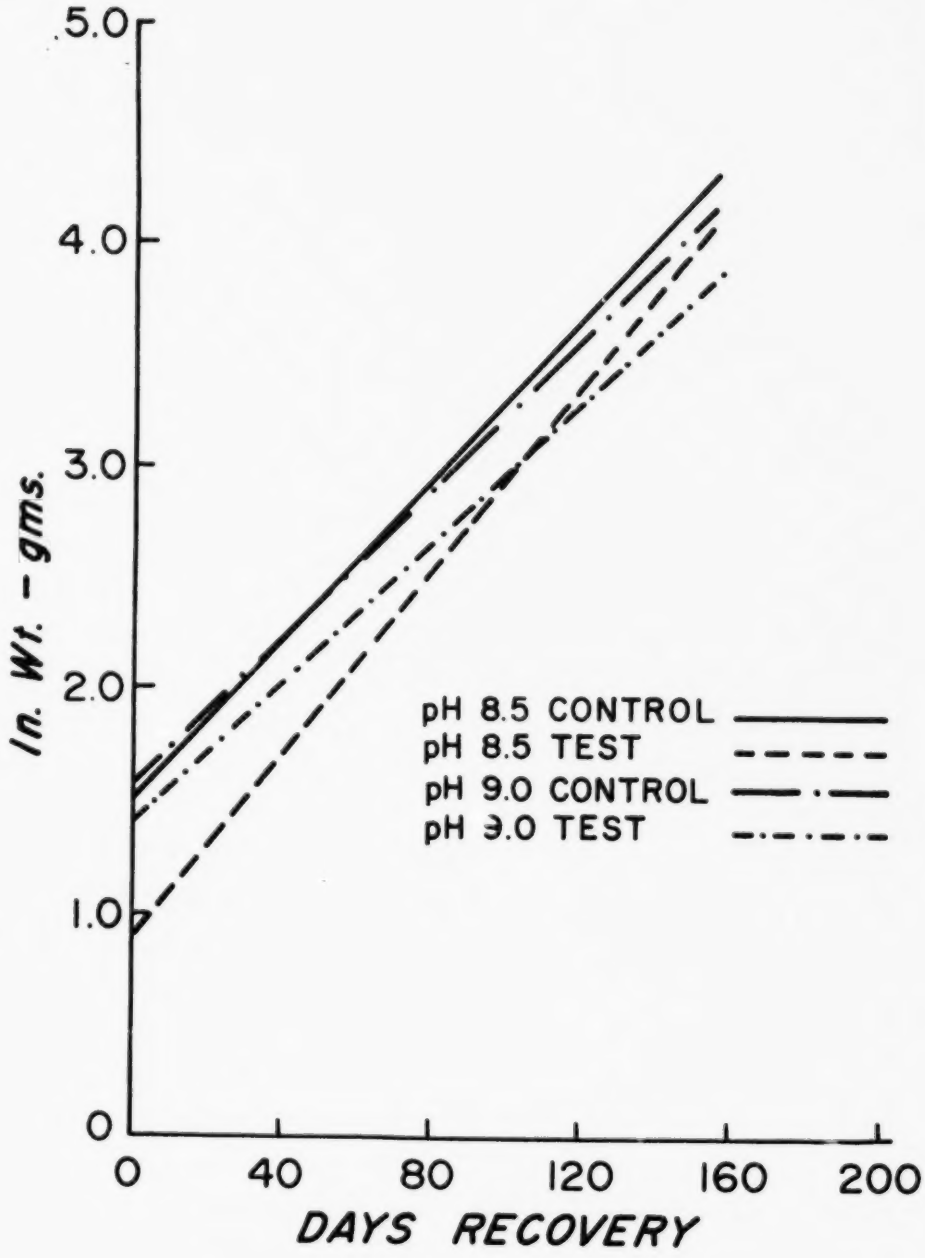

FIG. 7. Growth Rates of Fingerlings Recovering from Exposure to 5.2ppm Al at pH 8.5 and 9.0.

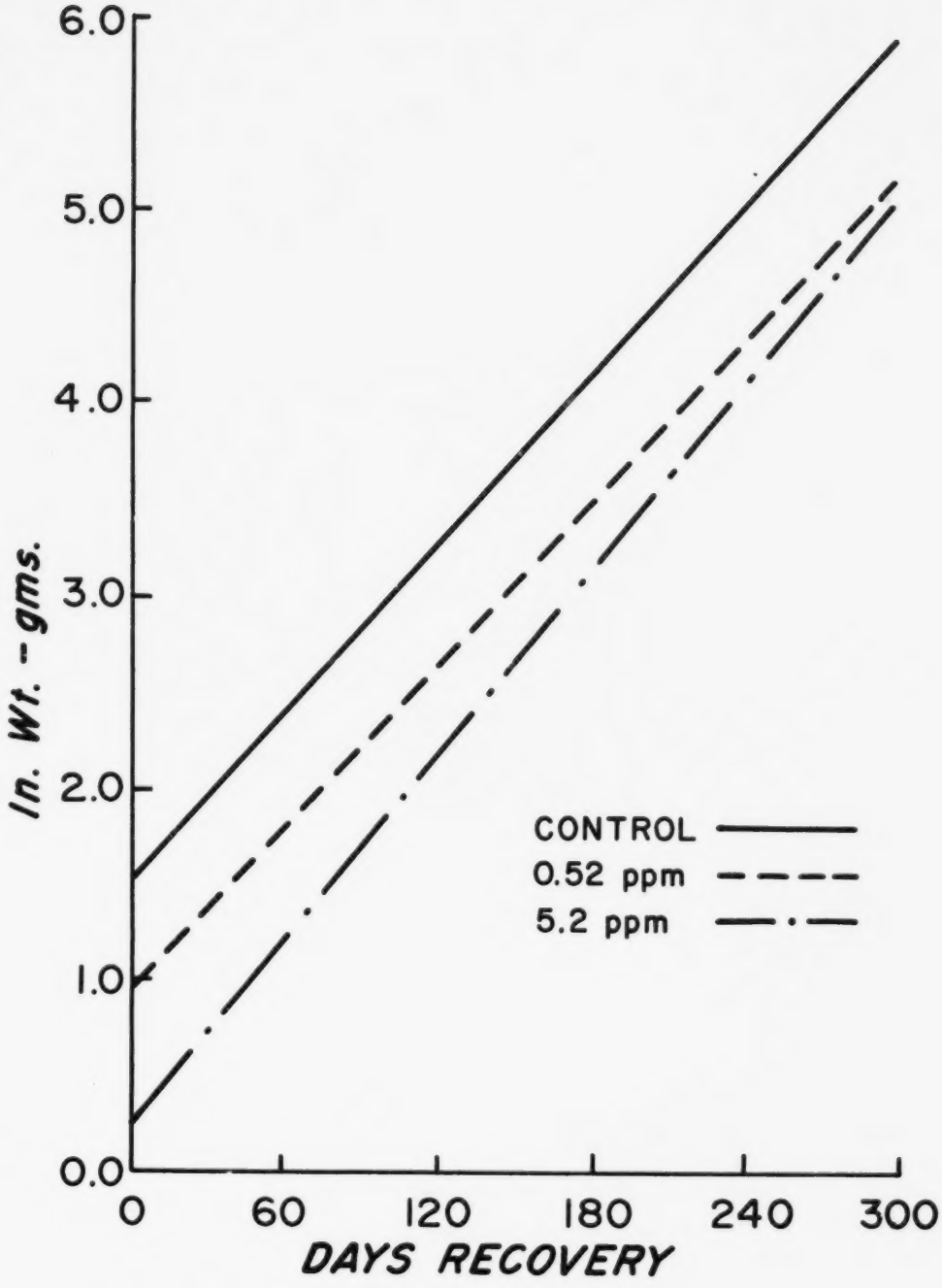

FIG. 8. Growth Rates of Fingerlings Recovering from Exposure to 0.52 and 5.2 ppm Al at pH 7.0.

Table 3

Comparison of Growth Rates During Recovery Periods

Hypothesis: $\text{Slope}_{\text{Test}} = \text{Slope}_{\text{Control}}$

Group	pH	Al ppm	Slope	r^2	t	
Control	8.5	-	.0167	.932		
Test	8.5	5.2	.0212	.973	.890	n.s.
Control	9.0	-	.0163	.930		
Test	9.0	5.2	.0158	.894	.097	n.s.
Control	7.0	-	.0145	.955		
Test	7.0	0.52	.0137	.973	.176	n.s.
Test	7.0	5.2	.0160	.971	.330	n.s.

Eggs and fry exposed in the submerged trays were studied in two experimental series: the first was made with controls and treatments at pH 9.0, and the second was made with controls at pH 8.0, with the 0.52 ppm Al at pH 7.0, and with the 5.2 ppm Al replicated at pH 7.0 and pH 8.0. Of first concern was the possible effects of aluminum on the fertilization. In both series all the control eggs were fertilized in the control water. One tray in each treatment trough was supplied eggs fertilized in control water and one tray was supplied with eggs fertilized in the appropriate aluminum concentration. Tables 4 and 5 present the data on egg mortality to the eyed stage for both series. None of the data would support any claim that aluminum toxicity reduced fertilization. In fact, in the first series the mortality to the eyed stage is lower for the eggs fertilized in the aluminum concentrations. The data in Tables 4 and 5 also confirm the high fertility of the eggs.

Although the figures for the egg and fry survival would seem to encourage the application of statistical comparisons, problems with the rearing and with maintaining constant pH's reduce the power of the early life history data. Consequently only general observations seem warranted. For example, total mortality from fertilization to termination for series one at standard pH 9.0 indicate a mortality of 74% for the controls, 60% for the 0.052 ppm Al, 55% for the 0.52 ppm Al, and 78% for the 5.20 ppm Al.

In the second series of the early life history exposures, total mortality from fertilization to termination was 50% for controls, 58% for the 0.520 ppm Al at pH 7.0, 68% for the 5.2 ppm Al at pH 7.0, and 92% for 5.2 ppm Al at pH 8.0.

A general conclusion is that dissolved aluminum is more toxic as evidenced by higher mortality of 5.2 ppm at pH 8 and pH 9, but that high concentrations of suspended aluminum (5.2 ppm, pH 7.0) are also nearly as toxic. The trend is the same as for the fingerling rainbows.

Table 4. Fertilization and early life history with controls and treatments at pH 9.0.

Trough	Treatment	Total Eggs at Start each Tray	Number Egg Mortality to Eyed Stage	Percent Mortality to Eyed Stage
1	Control	C 188	36	19
1	Control	C 201	36	17
2	Control	C 165	26	16
2	Control	C 190	42	22
3	0.052 *	A 276	8	3
3	0.052	C 196	21	11
4	0.052	A 211	2	1
4	0.052	C 183	35	19
5	0.520	A 337	3	1
5	0.520	C 171	32	19
6	0.520	A 301	0	0
6	0.520	C 174	22	13
7	5.200	A 226	12	5
7	5.200	C 220	35	16
8	5.200	A 335	1	1
8	5.200	C 198	29	15

* = ppm. aluminum concentration
C = fertilized in control water pH 9.0
A = fertilized in appropriate aluminum concentration pH 9.0

Table 5. Fertilization and early life history with controls at pH 8.0 (troughs 1 and 2), 0.52 ppm Al at pH 7.0 (troughs 3 and 4), 5.2 ppm Al at pH 7.0 (troughs 5 and 6), and 5.2 ppm Al at pH 8.0 (troughs 7 and 8).

Trough	Treatment	Total Eggs at Start each Tray	Number Egg Mortality to Eyed Stage	Percent Mortality to Eyed Stage
1	Control	C 299	37	12
1	Control	C 244	31	13
2	Control	C 274	34	12
2	Control	C 245	29	12
3	0.520*	A 289	24	8
3	0.520	C 225	22	10
4	0.520	A 230	14	6
4	0.520	C 262	20	8
5	5.200	A 276	20	7
5	5.200	C 214	20	9
6	5.200	A 216	12	6
6	5.200	C 245	36	15
7	5.200	A 257	29	11
7	5.200	C 212	25	11
8	5.200	A 152	15	10
8	5.200	C 232	31	13

* = ppm. aluminum concentration
C = fertilized in control water pH 8.0
A = fertilized in appropriate aluminum concentration and pH

SECTION VI

ACKNOWLEDGMENTS

The Department of Fishery and Wildlife Biology, Colorado State University, initiated the grant proposal, provided the facilities for the aluminum studies, and handled the administration. Dr. W. Harry Everhart, Professor and Chairman Fishery Major, was Project Director.

Mr. Robert Freeman, Graduate Student, designed the test facility, conducted the fingerling studies, and worked toward solving the aluminum chemistry problems.

Dr. Janet Osteryoung, Quantitative Chemist, was technical consultant for chemical problems. Mr. Kenneth Olsen, Graduate Student, Department of Chemistry, monitored the pH and prepared aluminum concentrations for the fry portion of the aluminum studies.

The support of the project by the Water Quality Office, Environmental Protection Agency, and assistance from Mr. J. Howard McCormick, Grant Project Officer, are acknowledged.

SECTION VII

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